

AMENDMENTS TO SPECIFICATION

Please amend the first four lines of the last paragraph on page 3 as follows:

In European Patent EP 0460480, entitled "Bacterin for the Treatment of Necrophorum Diseases and a Method for the Production Thereof, invented by Berg, a method is disclosed that uses whole-cell suspensions of *F. necrophorum* which have been inactivated using *p*-propiolactone β -propiolactone (BPL). The patent further discloses a method whereby the

Please amend the third paragraph on page 5 as follows:

The whole cell culture can be mixed with an amount of diluent, including adjuvants and saline solution or filler. An adjuvant is preferred because it allows for the delivery of a more anti-genically antigenically effective amount of the whole cell culture. Generally, the adjuvant allows for the use of a lesser amount of the bacterial culture in the vaccine. The most preferred adjuvant is oil based.

Please amend the last two lines on page 5 as follows:

enhance the anti-genicity antigenicity of the vaccine. Inactivating the cultures with formaldehyde allows for improved preservation of the killed bacteria, and using an adjuvant whose characteristics

Please amend the last paragraph of page 6 as follows:

The method for producing the vaccine is initiated by expressing any isolate of *F. necrophorum* bacteria. It is preferred that the isolate of *F. necrophorum* be virulent and obtained from a bovine. The isolate could be obtained from other host organisms, in particular other ruminants, but it is most preferred for the isolate to be taken from a bovine. To ensure optimum vaccine anti-genicity antigenicity, in accordance with the invention, a virulent strain of *F. necrophorum* bacteria is chosen for vaccine production. Determination of virulence can be made where a host organism exhibits acute signs of infection. The *F. necrophorum* bacteria can be biotype A (FNN), B [*F. necrophorum* subspecies *fundulifonne* (FNF)] or AB (FNN), any of which strain is believed to be available as an isolate for use in forming the present vaccine. More preferably, biotype A (FNN) *F. necrophorum* bacteria is selected because it tends to be the most virulent which will, in turn, most likely cause the strongest immune response when introduced into a host as a vaccine. In the most preferred method, the strain of *F. necrophorum* bacteria used is obtained from a bovine, and identified as ATCC Deposit No.: PTA-917

(ATCC, 10801 University Boulevard, Manassas, VA 20110-2209; deposited on Nov. 2, 1999). strain No. 021496, ATCC No. _____.

Please amend the first paragraph on page 9 as follows:

Termination of growth can be achieved using any method known to effectively terminate the growth of *F. necrophorum* bacteria or other similar bacteria while not significantly altering the protein or cellular products found within the bacterial culture. It is believed especially important not to use a method of terminating bacteria growth that will affect the bacteria cell wall integrity. Examples of suitable compositions for terminating growth include β -propiolactone, gluteraldehyde, and formaldehyde, ρ -propiolactone, gluteraldehyde and formaldehyde. In the preferred method, formaldehyde is used to terminate the growth of the bacteria because formaldehyde is believed to best maintain the anti-genicity antigenicity of the cell culture. It is hypothesized that the formaldehyde may in fact stabilize the antigens found in the cell culture. In the most preferred method, a 37% formaldehyde solution is used in an amount equal to about 0.4% by volume of the bacterial culture. Obviously, other amounts of formaldehyde or growth inactivating agent can be used.

Please amend the last two lines of page 9 and the top of page 10 as follows:

after vaccination. Representative examples of suitable adjuvants are aluminum salts, such as aluminum hydroxide and aluminum phosphate; polymers, such as POLYGEN POLYGEN™ (a preparation of low molecular weight, non-particulate co-polymer which can form cross-linkages in solution to become a high molecular weight gel), DEAE dextran, dextran sulfate, and methyacrylates; dimethylodecylammonium bromide; poxvirus proteins, such as Baypamune® (inactivated parapoxvirus ovis); Avirdine, Lipid A; oils, such as EMULSIGEN™ (an oil-in-water emulsion), EMULSIGEN PLUS™ (an oil-in-water emulsion), Suprlimm® (water-in-oil-in-water emulsion); animal oils, such as squalane or squalene; mineral oils, such as Drakeol® and Montanides®; vegetable oils, such as peanut oil; block co-polymers; triterpenoid glycosides, such as saponin, QuilA™ and QS21™; detergents, such as Tween-80™ and Pluronic™; bacterial component adjuvants, such as Corynebacterium, Propionibacterium and Mycobacterium; interleukins, monokines and interferons; liposomes; ISCOMs; synthetic glycopeptides, such as muryamyl dipeptides and derivatives thereof, cholera toxin; or combinations of the above. More preferably, the adjuvant is selected from the group consisting of oils, aluminum salts, polymers, dimethyldeodecylarnrnnonium bromide, poxvirus proteins, block co-polymers, triterpenoid glycosides, detergents and combinations thereof. Most

preferably, the adjuvant is an oil-based adjuvant, and in the most preferred method the adjuvant is an oil-based adjuvant which is produced under the name of Suprlmn® Suprlmn® oil, which is manufactured by ImmTech ImmTech Biologics, LLC, Bucyrus, KS 66013.

Please amend the last paragraph on page 10 as follows:

Once formed, the vaccine can be administered to any ruminant, but is preferably administered to a bovine, by any conventional procedure known, such as an intramuscular or subcutaneous injection. The subcutaneous injection is preferred because it is less likely to cause injection site lesions. The appropriate dosage of vaccine is determined primarily by the amount of bacteria and the anti-genicity antigenicity of the culture found in the vaccine. As such, any reasonable amount can be administered, with it being preferred that the dosage be 1 mL and 5 mL. A dosage of 2 mL is even more preferred. The smaller doses are preferred because they lessen the chance of lesions forming on the inoculated subject species. It is also

Please amend the seven lines at the top of page 15 as follows:

with Vaccine B and the third group were vaccinated with Vaccine C. The fourth group of calves vaccinated with Vaccine D, and the fifth group of calves were used as the unvaccinated controls. The animals received subcutaneous vaccinations on days 1 and 21 (2 mL doses with the starting culture equal to 1×10^8 CFU/mL). All of the calves were challenged at day 35 with a 5 ~~ml~~ ml dose of a 1×10^8 CFU/mL 8-hour virulent culture of *F. necrophorum* via portal skin. This concentration was previously used to infect similarly aged calves and caused liver abscesses.